NANOFIBER INCORPORATED INTRACANAL MEDICAMENTS AND ITS ANTIBACTERIAL EFFECT AGAINST ENTEROCOCCUS FAECALIS BIOFILM - AN INVITRO STUDY

Dissertation submitted to

THE TAMIL NADU DR M.G.R. MEDICAL UNIVERSITY

In partial fulfillment for the degree of

MASTER OF DENTAL SURGERY

BRANCH- IV

CONSERVATIVE DENTISTRY AND ENDODONTICS

2016- 2019

ENDORSEMENT BY THE H.O.D & PRINCIPAL / THE HEAD OF THE INSTITUTION

This is to certify that **Dr.P.SANJAY KUMAR**, Post Graduate student (2016–2019) in the Department of Conservative Dentistry and Endodontics, K.S.R. Institute of Dental Science and Research, has done this dissertation titled **"NANOFIBER INCORPORATED INTRACANAL MEDICAMENTS AND ITS ANTIBACTERIAL EFFECT AGAINST ENTEROCOCCUS FAECALIS BIOFILM – AN INVITRO STUDY"** under our guidance and supervision in partial fulfillment of the regulations laid down by **The Tamil Nadu Dr. M.G.R. Medical University**, Chennai – 600 032 for **M.D.S.,** (Branch – IV) **CONSERVATIVE DENTISTRY AND ENDODONTICS** degree examination.

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TITLE OF DISSERTATION	**Nanofiber incorporated intracanal medicaments and its antibacterial effect against enterococcus faecalis biofilm – an invitro study**
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APPENDIX III

This is to certify that this dissertation work titled "**NANOFIBER INCORPORATED INTRACANAL MEDICAMENTS AND ITS ANTIBACTERIAL EFFECT AGAINST ENTEROCOCCUS FAECALIS BIOFILM – AN INVITRO STUDY**" of the candidate **DR.P.SANJAY KUMAR** with registration number **241617403** for the award of "**Master of Dental Surgery**" in the branch of **Conservative Dentistry and Endodontics** . I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **6 %** percentage of plagiarism in the dissertation.

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INTRODUCTION

The success of endodontic therapy depends on complete disinfection of root canal system which can be accomplished by thorough chemomechanical preparation and three dimensional obturation of the root canal system.[1] Infected teeth have formation of bacterial biofilm on canal walls, lateral canals, in the isthumus, inside dentinal tubules and on apical extra radicular root surface.[2]

Endodontic infection consists of between 1 and 12 bacterial species. This colonization comprises of mostly facultative and strict anaerobic bacteria which have the ability to proliferate via interactions among bacterial cell proteins that establish a complex spatial structure known as biofilm. Several bacterial species have been identified including Enterococcus faecalis and Streptococcus sanguinis Meanwhile, a recent study found Actinomyces naeslundii to be the most prevalent organism in traumatized immature permanent teeth with necrotic pulps[3].

Enterococcus faecalis is a mushroom shaped microorganism seen in dentinal tubules, isthumus, rami, and in a lateral canals. Enterococcus faecalis is the most commonly seen microorganism in asymptomatic persistent infections and also poorly endodontic treated teeth . Enterococcus faecalis is the predominant bacteria that causes root canal failuers. It is a facultative anaerobic bacteria. Enterococcus faecalis is present in a small proportion of the flora in canals that are untreated. Enterococcus faecalis biofilms are resistant against antibodies, antimicrobial agents and phagocytes.[4]

A Triple antibiotic mixture of metronidazole, ciprofloxacin, and minocycline is used for root canal disinfection. When using a mixture of metronidazole, ciprofloxacin, and minocycline bacterial elimination is seen in the deep root canal dentin. Metronidazole being bactericidal,

Minocycline being bacteriostatic and Ciprofloxacin being a bactericidal broad spectrum synthetic quinolone, triple antibiotic paste is able to eradicate both gram positive aerobes and anaerobic bacteria. Antibiotic ineffectiveness in systemic route of administration has led to the intra canal application to increase its efficacy.[5]

Calcium hydroxide is the most commonly used intracanal medicament for disinfection. Calcium hydroxide is most widely used because of its alkaline Ph.[6] Intracanal medicaments are used within the canal because of their antimicrobial activity, their ability to neutralize the tissue remnants within the canal and because of their ability to prevent and control pain after treatment.[7]

Poly vinyl pyrrolidone is an important synthetic polymer. It is a water soluble polymer used for biomedical and pharmaceutical applications due to its non toxic, noncarcinogenic and bioadhesive properties. Poly vinyl pyrrolidone is one of the most important agents in cosmetic market used in skin care products, shampoos, lipsticks, deodorants, sunscreen products due to its low chemical toxicity and biocompatibility. This polymer nanofiber can be easily prepared by electrospinning. A High electric field is necessary for electrospinng. This high electric field is to draws a polymer solution from the tip of a capillary towards the collector. The applied voltage causes a jet of the solution to be drawn toward a grounded collector. The fine jets form nanosized polymeric fibers after they dry up and they are collected as a web like mass.[8]

Electrospinning is a nanotechnology technique capable of fabricating antibiotic-eluting polymer nanofibers for use in drug delivery. Antibiotic nanofibers may provide clinical benefit because of its substantial antimicrobial properties.[9].

In this present study, we have used the triple antibiotic paste and calcium hydroxide with and without nanofiber as intracanal medicaments to compare their antimicrobial property against enterococcus faecalis.

AIM

The aim of this in vitro study was to evaluate and compare the antibacterial effect of calcium hydroxide and triple antibiotic paste with and without nanofiber against enterococcus faecalis biofilms.

OBJECTIVES

To compare the antibacterial effect of calcium hydroxide and triple antibiotic paste with and without nanofiber against enterococcus faecalis biofilms using confocal laser scanning microscope

MANAVALAN MADHANA MADHUBALA ET AL (2011) evaluated and compared the antimicrobial activity of calcium hydroxide, triantibiotic paste and ethanol extract of propolis as an intra canal medicament on enterococcus faecalis. In their study they used 120 extracted human permanent incisors. The incisors were decoronated and chemomechanical preparation was performed. The samples were randomly divided into 5 groups. Group 1:calcium hydroxide, Group 2: tri antibiotic mixture, Group 3:propolis, Group 4:ethanol and Group 5: saline (control group). The antibacterial effectiveness of the different intra canal medicaments was recorded by determining the percentage of reduction in colony counts at 1, 2, and 7 days. They concluded that propolis was more effective than triantibiotic mixture against enterococcus faecalis at a 2 day time period, and both were equally effective at 7 days period.[10]

ALAA H.A.SABRAH ET AL (2015) evaluated the effect of various dilutions of antibiotic medicaments used in endodontic regeneration on survival of human dental pulp stem cells and determined their antibacterial efficacy against Enterococcus faecalis biofilm. The cytotoxic and antibacterial effects of different triple and double antibiotic paste dilutions (0.125, 0.25, 0.5, 1 and 10 mg/ ml) were tested against enterococcus faecalis biofilm and dental pulp stem cells. Bacterial biofilm was exposed to antibiotic dilutions for 3 days. After 3 days the biofilm was collected, spiral plated, and the number of bacterial colony forming units were determined. The cytotoxic effect was evaluated by performing the lactate dehydrogenase assay and cell viability assays. They concluded that 0.125 mg/ml of triple antibiotic paste and double antibiotic paste did not negatively affect the viability of dental pulp stem cells. This concentration was not enough to completely eradicate enterococcus faecalis. Minimal antibiotic concentration should be considered during endodontic regeneration procedures.[11]

BLAKE T. PRATHER ET AL (2014) compared the effects of triple antibiotic paste and modified triple antibiotic paste concentrations on the microhardness and chemical structure of radicular dentine. Single rooted matured premolars were selected to perform the study. A cervical 5mm root cylinder was obtained from each tooth. The coronal side of each cylinder was levelled with the surface of the acrylic block in order to obtain easy access to the root canal. Group1-1g/ml triple antibiotic paste, Group2-1g/ml modified triple antibiotic paste, Group3-1mg/ml triple antibiotic paste Group4-1g/ml modified triple antibiotic paste and for control sterile water was applied to the canal. Cylinders were stored at 100% relative humidity for 4 weeks. Cervical root cylinders measuring 5mm were subjected to a microhardness test before and after treatment, and were examined using attenuated total reflection fourier transform infrared spectroscopy. Their result showed that 1mg/ml methylcellulose based triple antibiotic paste and modified triple antibiotic paste caused significantly less reduction in the microhardness when compared to other treatment groups and untreated control dentin. The use of 1mg/ml triple antibiotic paste and modified triple antibiotic paste can remarkably minimize the reduction in root microhardness when compared to the presently used concentration of antibiotic medicaments.[12]

ALAA H.A. SABRAH ET AL (2013) in their study, used triple antibiotic paste, double antibiotic paste and calcium hydroxide paste against enterococcus faecalis and porphyromonas gingivalis biofilm. The pastes were prepared by using mixing 16mg of Calcium hydroxide powder with 1ml distilled water and triple antibiotic powder (metronidazole, ciprofloxacin, minocycline) in 3ml distilled water and Double antibiotic powder (metronidazole,ciprofloxacin) in 3ml distilled water. All the antibiotics were stirred for 4 hours and centrifuged at 3000rpm for 15 minutes.Enterococcus faecalis and porphyromonas gingivalis were determined by 2 fold dilution method and then all the

medicated solutions were placed for 24 hours in sterile 96 well flat bottom microtiter plates. They concluded that both triple antibiotic paste and double antibiotic paste were more effective than calciumhydroxide against enterococcus faecalis and porphyromonas bacteria. It was also observed that triple antibiotic paste causes discolorations when compared with double antibiotic paste.[13]

MOHAMMED FROUGH REYHANI ET AL (2015) evaluated the antimicrobial effects of different concentrations of triple antibiotic paste at 1,2,3 and 4 week intervals on mature enterococcus faecalis biofilm. 287 extracted human central incisors were infected with enterococcus faecalis . The root canal space was filled with one of the 0.01, 0.1, 1, 10, 100, and 1000mg/ml concentartions of triple antibiotic paste and the control group was filled with normal saline. After 24 hours gates glidden drills were used to collect 10mg of dentin chips from the root canal walls. The dentine chips were transferred into test tubes containing 2ml of sterile physiologic serum and mixed for 20 seconds using whiriling movements. 100ml of each dilution was added to 30 plates of mueller hinton cultures and incubated at 37°c for48 hours. Classic colony forming unit counting technique was used to count enterococcus faecalis colonies that were retrieved. They concluded that use of lower concentrations of triple antibiotic paste might decrease the tooth discoloration potential and at the same time it also increases the potential of regeneration due to a decrease in the negative effects on the stem cells in the area. Bacterial biofilms might be erradicated from the root canal space when this medicament is placed in canal for one week.[14]

ALIREZA ADL ET AL 2014 compared the ability of triple antibiotic paste to calcium hydroxide in disinfecting dentinal tubules. 60 extracted single rooted human teeth were selected. Portions measuring 6mm length from the middle third of each root were obtained by cutting the coronal and apical parts of the roots. Gates Glidden drills were used to enlarge the canals. For contamination of the specimens , each block was transferred to a pre-sterilized microcentrifuge tube containing 1ml of the TS broth and then 50 µl of an inoculum of Enterococcus faecalis was added to each tube. Every 2 days the blocks were transferred to fresh TSB containing Enterococcus faecalis during a period of 3 weeks. They concluded that the triple antibiotic paste group was more effective in disinfecting the canal against enterococcus faecalis when compared to the chlorhexidine group.[15]

C.MANIGLIA FERREIRA ET AL 2016 compared different intracanal medicaments to assess the antimicrobial effectiveness against enterococcus faecalis. The medicaments assessed in this study were calcium hydroxide in different mediums, several combinations of triple antibiotic paste and double antibiotic paste. Group 1-triple antibiotic paste (metronidazole,ciprofloxacin,amoxicillin) Group 2- double antibiotic paste (amoxicillin, metronidazole) Group3- double antibiotic paste with calcium hydroxide, Group 4-calcium hydroxide with 0.9% saline solution, Group 5- calcium hydroxide with chlorhexidine2% gel, Group 6- Double antibiotic paste (metronidazole,ciprofloxacin), Group 7 -double antibiotic paste with zinc oxide eugenol, Group 8-Double antibiotic paste with calcium hydroxide and the control group (0.9 saline solution). They concluded that triple antibiotic paste (metronidazole, ciprofloxacin, amoxicillin) and Double antibiotic paste (amoxicillin, metronidazole) showed more antibacterial efficacy against enterococcus faecalis. These medicaments remained active for 30 days when used as intra canal dressings in necrotic immature teeth.[16]

RONALD ORDINOLA ZAPATA ET AL 2013 had performed a study to evaluate the antimicrobial activity of calcium hydroxide, 2% chlorhexidine gel and triantibiotic paste by using an intraorally infected dentin biofilm model. Forty sterile bovine dentin sections (2×2×2mm) were taken. The dentin samples were placed into the cavities of a hawleys orthodontic device with sticky wax. The dentin surface in contact with the oral cavity was fixed 1mm above the surface to favour the accumulation of plaque. Each sample was incubated in 2ml brain heart infusion medium at 37°C for 24 hours under aerobic conditions. The infected dentin samples were immersed in medicaments for 7 days. After 7 days the medicaments were washed with saline solution. Five treated dentin blocks were immediately evaluated by confocal analysis, and 5 blocks were immersed in 2ml fresh BHL and incubated at 37°C. These last samples were evaluated after 24 hours of the elimination of the antimicrobial substances. They concluded that triantibiotic paste was most effective at killing bacteria in the biofilms on the intraorally infected dentin model in comparison with 2% chlorhexidine gel and calcium hydroxide paste.[17]

D.A.ATTIA ET AL 2015 compared the antimicrobial effect of calcium hydroxide paste, chlorhexidine gluconate gel and Antibiotic corticosteroid paste against Enterococcus faecalis, Streptococcus mutans and Candida albicans in root canal lumen and radicular dentin. Eighty four extracted single rooted teeth were used in this study. The canal length was standardized at 15mm approximately. The diameter of the root canals were standardized by selecting all the canals with initial apical file size no.25. Biomechanical preparation and irrigation was performed. Each root was placed in a closed test tube containing 4ml of brain heart infusion broth. It was sterilized by autoclaving at 121°c for 20 min and incubated for 24 hours at

37°C. 2ml of the sterile brain heart infusion broth from each tube was replaced by 2ml of the prepared mixed bacterial suspension and then the test tubes were closed and incubated at 37°C for14 days. They were grouped as Group 1-calcium hydroxide paste, Group 2-chlorhexidine gel, Group 3 -corticosteroid antibiotic paste and Group 4-sterile physiologic saline (control group). Orifices of the canals were sealed with dental modelling wax and placed in humid sterile gauze in closed sterile Petri dishes. All the specimens were incubated for 7 days at 37°C. After the completion of the incubation period the wax seal and intra canal medicaments were removed. Microbial suspension was placed on the three media specific for the growth of the tested microorganisms. The growing colonies were counted and recorded as colony forming units. They concluded that chlorhexidine was the best medicament used to eliminate the three different tested organisms at the two experimental sites, root canal lumen and radicular dentin. Streptococcus mutans was the most sensitive microorganism to calcium hydroxide, chlorhexidine and triple antibiotic paste. Candida albicans was the most resistant microorganism. Enterococcus faecalis was more susceptible to chlorhexidine than the other medications.[18]

MARIA TANUMIHARDJA Et al 2015 in their study used intracanal triple antibiotic paste dressing to remove remaining bacteria while performing root canal treatment in patients with chronic apical periodontitis. They evaluated the antimicrobial effects of triantibiotic paste from the exudates of human root canals. 24 necrotic root canals were taken in this study and chemo mechanical preparation done. Intra canal medication with calcium hydroxide paste was given for seven days. After 7 days calcium hydroxide paste was removed from the canals. The canals were irrigated and one group of samples were medicated with triple antibiotic paste. The other group was remedicated with calcium hydroxide paste. They

concluded that triantibiotic paste showed better antimicrobial effect over calcium hydroxide paste and it also prevented the growth of bacteria.[19]

ABBAS ABBASZADEGAN Et al 2016 investigated the chemical constituents of cinnamomum zeylanicum essential oil. They compared the antimicrobial activity of cinnamomum zeylanicum with triple antibiotic paste and calcium hydroxide on planktonic and biofilm enterococcus faecalis. They also compared the cytotoxicity of these medicaments on L929 fibroblasts. Gas chromatography mass spectrometry analysis was performed by using agilent 7890 gas chromatograph with a mass detector. A total of 108 human teeth were infected with Enterococcus faecalis and the treated with medicaments for 1,7 and 14 days. Cytotoxicity was checked by exposing L929 fibroblasts to the medicaments. Cinnamaldehyde was the main constituent of cinnamomum zeylanicum essential oil. Triple antibiotic paste and cinnamomum zeylanicum essential oil completely eradicated planktonic and biofilm Enterococcus faecalis. Planktonic and biofilm Enterococcus faecalis were eradicated after 4 and 24 hours and after 7 days and 14 days respectively. When compared to triple antibiotic paste, Calcium hydroxide failed to completely eradicate Enterococcus faecalis after 24 hours.[20]

SARMAD M. ALYAS ET AL 2016 evaluated the direct and indirect antibacterial effects of various concentrations of triple antibiotic paste loaded into a methylcellulose system. A total of 180 intact human permanent teeth were used in this study. Enterococcus faecalis was grown on sterilized dentin blocks and treated with clinically used triple antibiotic paste(1000mg/ml), low concentrations of methylcellulose based triple antibiotic paste (100,10, 1mg/ml), placebo paste or 1.5% sodium hypochlorite. They concluded that all

concentrations of direct triple antibiotic paste eradicated the biofilms in comparison with placebo paste,while10 mg/ml of triple antibiotic paste or higher provided substantial residual antibacterial effects. Dentin treated with 1mg/ml of triple antibiotic paste or 1.5% sodium hypochlorite did not provide substantial residual antibacterial effects. Dentin pretreated with 10mg/ml of triple antibiotic paste or higher concentration showed increased residual antibacterial effects and can be used in endodontic regeneration procedures.[21]

ALIREZA ADL et al 2012 compared the antimicrobial ability of triple antibiotic paste and its components against calcium hydroxide mixtures. Efficacy of medicaments in removing enterococcus faecalis is determined by an agar well diffusion assay and MIC method. The groups consisted of triple antibiotic paste with saline or 2% chlorhexidine, metronidazole, ciprofloxacin, minocycline antibiotics separately with saline and calcium hydroxide paste with normal saline or 2% chlorhexidine. The diameter of the growth inhibition zones for each group were recorded and compared. They concluded that triple antibiotic paste with either 2% chlorhexidine or saline would be a better medicament against enterococcus faecalis. Among the three components minocycline has the highest antibacterial effect.[22]

GARIMA TIWARI et al 2018 conducted a study using plant essential oils and observed that it possesses strong antimicrobial activity that can be harnessed to remove enterococcus faecalis from the root canal environment. Enterococcus faecalis was cultured in BHI broth and incubated at 37°C for 24 hours. The bacterial suspension was inoculated on 30 mueller hinton agar plates and were filled with medicaments. They were incubated at 37°C for 72 hours. They were grouped as Group A-Aniba rosaeodora and calcium hydroxide, Group B-Origanum vulgare and calcium hydroxide, Group C-Calcium hydroxide paste and Group D-

triple antibiotic paste. They concluded that oregano oil and rosewood oil in combination with calcium hydroxide can be used as intracanal medicaments to increase the antimicrobial effect and retention within the root canal.[23]

SHIBHA MEHTA et al 2018 compared the antimicrobial efficacy of triple antibiotic paste and a proton pump inhibitor in combination with calcium hydroxide against enterococcus faecalis and candida albicans. Both bacteria were subcultured and inoculated at 37° overnight. The organisms were treated with different dilutions of triple antibiotic paste. Group 1-triple antibiotic paste 25µg/ml, Group 2- calcium hydroxide 16mg/ml, Group 3a- calcium hydroxide 16mg+omeprazole 2mg/ml, Group 3b- calcium hydroxide 16mg/ml+omeprazole 4mg/ml for 24, 48 and 72 hours in sterile uncoated 96 well microtiter plates. They concluded that the proton pump inhibitor increases the antibacterial efficacy of calcium hydroxide against enterococcus faecalis and candida albicans. Triple antibiotic paste showed better antibacterial efficacy than calcium hydroxide + proton inhibitor.[24]

MANJEET KAUR et al 2017 observed the potential of combinations of amoxicillin+metronidazole, amoxicillin clavulanic acid+ metronidazole, amoxicillin and cloxacillin+metronidazole over triple antibiotic paste. Fifty single rooted teeth were taken and standardized to 13mm length from the apex. Bacteria was cultured on the blood agar plate and at the same time fresh antibiotic paste combinations were prepared. Triple antibiotic paste was placed in one group. Different combinations of antibiotic powder + propylene glycol were placed in the root canal and then incubated and observed at 24, 48, 72 hours. The triple antibiotic paste showed a zone of inhibition which was lesser when compared to, amoxicillin and clavulanic acid combination along with metronidazole .[25]

TRIVENI M NALAWADE et al 2016 determined the antibacterial effectiveness of endodontic medicaments and various vehicles using an agar well diffusion assay. The medicaments used were double antibiotic paste, modified double antibiotic paste, 2% chlorhexidine gel used with vehicles like polyethylene glycol, propylene glycol, combination of propylene glycol with polyethylene glycol and glycerine. The antimicrobial activity against Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis, Porphyromonas gingivalis and Escherichia coli were determined. The vehicles used to form the pastes play a supportive role by forming the appropriate consistency for placement and may considerably influence their chemical characteristics like their solubility and diffusion. They concluded that 2% chlorhexidine gluconate and modified double antibiotic paste can replace double and triple antibiotic paste as an intra canal medicament with chlorhexidine to assist in obtaining bactericidal action, substantivity, biocompatibility, low toxicity, and lesser chances of developing resistance.[26]

KRITTIKA RAVI et al 2017 compared the antimicrobial activity of calcium hydroxide gel, chlorhexidine gel, triple antibiotic paste and double antibiotic paste as intra canal medicaments against Enterococcus faecalis. Twenty four human single rooted teeth were decoronated. Chemomechanical preparation was performed. They were grouped as Group1- calcium hydroxide gel, Group2- chlorhexidine gel, Group3- triple antibiotic paste and Group4-double antibiotic paste. The antibacterial effectiveness of all the intra canal medicaments were seen at end of 14 days. They concluded that triple antibiotic was more effective than the other groups against Enterococcus faecalis after 14 days.[27]

SHOLEH GHABRAEI et al 2017 determined the minimum duration of application of triple antibiotic paste required for elimination of Enterococcus faecalis from the root canal and its minimum inhibitory concentration and minimum bactericidal concentration. 34 single rooted teeth were inoculated with Enterococcus faecalis after chemomechanical preparation. On the prepared specimens 4 gram of triple antibiotic paste was applied as intra canal medicament. The teeth were sectioned longitudinally. Dentin chips were collected and checked to determine the count of bacterial colonies. They concluded that after 7 days of intra canal medication the concentration of triple antibiotic paste used was found to be 5×10^4 times its minimum inhibitory concentration. They observed that a lower concentration of triple antibiotic paste can be used to prevent coronal discoloration of teeth.[28]

PRATIBHA AHIRWAR et al 2018 evaluated the aerobic and anaerobic antimicrobial effect of Ocimum Sanctum essential oil and triple antibiotic paste by collecting microbiological samples of root canals. 40 patients were selected and divided into 2 groups. O.sanctum group and triple antibiotic group. These samples were cultured in aerobic and anaerobic environment and later colony forming units were counted. They concluded that Ocimum sanctum can be used in cases of long lasting infection owing to its antibacterial effect and anti-inflammatory potential as an intracanal medicament in deciduous teeth.[29]

JEISON B. CARBAJAL MEJIA et al 2015 determined the survival of enterococcus faecalis after a 14 day exposure to 1% cetrimide, 2% chlorhexidine gel, calcium hydroxide paste, and triantibiotic paste in infected dentin models. 75 single rooted human teeth were taken. The groups taken were Group1- saline solution (negative group), Group2- Calcium hydroxide, Group3- 2% chlorhexidine gel,, Group 4-triantibiotic paste, and Group5- 1%

cetrimide. The specimens were collected and stained with SYTO 9 of fluorescence microscopy to view viable cells. They concluded that both the medicaments 1% cetrimide and triple antibiotic paste considerably reduded the viability of Enterococcus faecalis in dentine when compared with the other medicaments used.[30]

J.M.V.M. DE LUCENA et al 2013 performed an invitro study to check the viability of enterococcus faecalis in infected human root dentin after exposure to chlorhexidine and octenidine intra canal medicaments.40 single rooted tooth were taken in this study. They were grouped as Group1- calcium hydroxide paste, Group2- chlorhexidine gel, Group3- chlorhexidine / gutta-percha points and Group4- octenidine gel. All the samples were incubated for 4 weeks . Enterococcus faecalis viability was checked by two fluorescent dyes. This was done in order to check the viable bacteria and colony forming units. They concluded that both chlorhexidine and octenidine based intracanal medicaments were effective in decreasing viability of Enterococcus faecalis. When compared to chlorhexidine, octenidine showed favourable results.[31]

JEISON B. CARBAJAL MEJIA et al 2013 determined the effect of calcium hydroxide , 2% chlorhexidine gel and propolis against Enterococcus faecalis and candida albicans. This was checked in infected dentin models at two different depths (100 and 200µm) after 14 days of application. In this study 120 single rooted teeth were taken and biomechanical preparation done and the tooth were sterilized . 120 samples were divided into four two equal groups. 60 samples were infected with Enterococcus faecalis and 60 samples with candida albicans. Each group was subdivided into 4 groups. Group1-saline (negative control), Group2-calcium hydroxide paste, Group3-chlorhexidine 2% and Group 4- propolis. They

concluded that chlorhexidine had the highest antifungal activity on candida albicans, but chlorhexidine and propolis were effective against Enterococcus faecalis.[32]

SHARMILA DEVARAJ et al 2016 investigated the effect of five intracanal medicaments against mature Enterococcus faecalis biofilm. 110 single rooted mandibular premolars take for this study. They were divided into 5 groups namely Group1- light activated curcumin, Group2- triple antibiotic paste, Group3- double antibiotic paste, Group4-chlorhexidine,Group 5-calcium hydroxide paste. Enterococcus faecalis was placed on sterile brain heart infusion broth and incubated at 37°C for 24 hours. The biofilm percentage of live/dead bacteria within root canal and dentinal tubules was analysed by confocal microscopy. They concluded that photoactivated curcumin showed the highest biofilm eradication when compared with triple antibiotic paste but there was no significant difference. Curcumin, triple antibiotic paste, double antibiotic paste showed a significant reduction of colony forming units/mL.[33]

MARYAM JAVIDI et al 2011 evaluated the effect of calcium hydroxide paste on the eradication of intraluminal and intratubular Enterococcus faecalis.36 single rooted human teeth were taken. The teeth were decoronated and the length was standardized to 15mm. Out of which 15 tooth were incubated and evaluated after one day. Another 15 samples were incubated and then the evaluated for seven days. Paper points and Gates Glidden burs were used to obtain intraluminal and intratubular Enterococcus faecalis. They concluded that calcium hydroxide cannot completely eradicate Enterococcus faecalis from root canals. Calcium hydroxide showed the same antibacterial effect on the intraluminal and intratubular Enterococcus faecalis.[34]

AR PRABHAKAR et al 2013 determined and checked the effect of turmeric extract and 2% chlorhexidine gel as an intracanal medicament against enterococcus faecalis and its effect on the microhardness of root dentin. 70 single rooted human teeth were taken. They were divided as Group1- calcium hydroxide ,Group2- 2% chlorhexidine, Group3- turmeric extract, Group4 -saline and Group 5- negative control. All the infected dentin blocks were kept for 21 days in Enterococcus faecalis. After treatment with medicaments, Vickers hardness indentation machine was used to check the microhardness of dentin blocks. They concluded that the turmeric extract has better activity with no difference on the microhardness of root canal dentine and hence it can be used as an intra canal medicament.[35]

MARYAM JAVIDI et al 2013 investigated the effect of calcium hydroxide paste with or without a silver nanoparticle suspension to eradicate Enterococcus faecalis from root canal. 66 single rooted human teeth were used in this study. All the tooth were contaminated with Enterococcus faecalis and were treated with 10% calcium hydroxide paste alone, calcium hydroxide paste with nanosilver or sterile water . All samples were obtained with paper points and gates glidden burs on the first and seventh day. The colony forming units were determined. They concluded that combination of calcium hydroxide and nanosilver as an intracanal medicament considerably decreases the number of enterococcus faecalis in the root canal.[36]

ASHIK ALI LAKHANI et al 2017 investigated and compared the antimicrobial activity of triple antibiotic paste, moxifloxacin, calcium hydroxide paste and 2% chlorhexidine gel in eradicating Enterococcus faecalis. 75 root blocks were taken from single rooted human tooth. They were grouped as Group1- saline, Group2- calcium hydroxide, Group3- 2%

chlorhexidine gel, Group4- triple antibiotic paste and Group5- moxifloxacin. All the dentin blocks were contaminated with Enterococcus faecalis for 21 days. All dentin debris were obtained at end of 1st, 7th and 10th day. Bacterial load was checked by counting the number of colony forming units. They observed that 2% chlorhexidine gel was the most effective intra medicament against Enterococcus faecalis in infected root dentin.[37]

ANUJ BHARDWAJ et al 2012 investigated and compared the antibacterial effect of natural extracts of morinda citrifolia, papain, and aloe vera, 2% chlorhexidine gel and calcium hydroxide against enterococcus faecalis. 180 extracted single rooted teeth were used in this study. They were grouped as Group1- saline, Group2- calcium hydroxide paste, Group3- papain gel, Group4- M.citrifolia gel, Group5- Aloe vera gel and Group6- 2% chlorhexidine gel. Dentin shavings were collected at 2 depths of 200 and 400µm. Colony forming units were determined after 1,3 and 5 days. They concluded that among the natural intracanal medicaments M.citrifolia gel showed good inhibition up to the 5th day followed by aloe vera gel and papain gel. In this study Chlorhexidine gel showed the highest antimicrobial activity against enterococcus faecalis, whereas calcium hydroxide showed the least antimicrobial activity.[38]

HEMANSHI KUMAR ET AL 2018 determined the antimicrobial effect of curcuma longa (turmeric-T_1-10%, T_2-20%) Tachyspermum ammi (ajwain- A_1-10% - A_2-20%), chlorhexidine gluconate gel (hexigel), and calcium hydroxide (10%) as an intra canal medicament against enterococcus faecalis. Agar plates were prepared by using brain heart infusion agar. Enterococcus faecalis was cultured in brain heart infusion broth at 37°c for 24 hours. All the intra canal medicaments were inoculated in agar plates. Plates were inoculated for 72 hours at

37°C the microbial and zones of inhibition were recorded. They concluded that curcuma longa (T_2-20%) was effective in eradicating Enterococcus faecalis. They suggested that it can be used as an intracanal medicament in endodontic retreatment.[39]

KANDASWAMY ET AL 2010 checked the antimicrobial activity of 2% chlorhexidine gel, propolis, morinda citrifolia juice, 2% povidone iodine and calcium hydroxide against enterococcus faecalis in infected root canal dentin at two different depths (200µm and 400µm) and three time intervals (1,3 and5 days).Total of 180 human teeth were taken and infected for 21 days with enterococcus faecalis. They were grouped as Group1- saline (negative control), Group2- propolis, Group3- morinda citrifolia juice, Group4- 2% Povidone Iodine, Group5 – 2% chlorhexidine gel and Group6 – calcium hydroxide. At the end of 1, 3and 5 days the remaining live bacteria were assessed. Dentin chips were taken at two different depths (200µm and 400µm) and the total number of colony forming units were checked. They concluded that propolis and morinda citrifolia juice were the most effective medicaments against enterococcus faecalis in root canal. When compared to the other intra canal medicaments used in this study.[40]

GUVEN KAYAOGLU ET AL 2011 determined antibacterial activity of enterococcus faecalis by using two propolis samples, chlorhexidine, and calcium hydroxide in a dentin block model. Root Canal dentin was sampled after 1 or 7 days by using a standard bur size. The dentinal shavings were vortexed vigorously in phosphate buffered saline, and aliquots were cultured on tryptone soy agar plates. Colonies forming were counted after 2 days of incubation. They observed that propolis did not have a greater performance when compared with chlorhexidine in eradicating Enterococcus faecalis.[41]

JULIANA Y. NAGATA ET AL 2014 checked the microbial composition of traumatized immature teeth and checked their reduction during different stages of revascularization procedures performed with two intra canal medicaments. They were grouped as Group1- triple antibiotic paste, Group2- calcium hydroxide + 2%chlorhexidine. Cultivable bacteria recovered from five stages were counted and identified by means of polymerase chain reaction assay. They concluded that bacterial reduction was promoted by irrigation solutions and the revascularization protocols that used the tested intracanal medicaments were effective in reducing live bacteria in necrotic immature teeth.[42]

YADAV ET AL 2018 evaluated the antimicrobial effect of calcium hydroxide, chlorhexidine gel, and curcumin based formulation against enterococcus faecalis. 30 single rooted human teeth were taken. The teeth were decoronated, a root length of 14mm was maintained and placed in a sterile Eppendorf tube. They concluded that chlorhexidine gel group showed better antimicrobial properties against enterococcus faecalis than other the groups in this study.[43]

AGRIMA VASUDEVA ET AL 2017 determined the antibacterial effect of 2% chlorhexidine gel, Honey, Aloe vera gel, Curcuma longa, Propolis gel and Calcium hydroxide against enterococcus faecalis in infected dentinal tubules. A Total of 210 mandibular first premolars were infected with enterococcus faecalis for 21 days. At the end of one, three, and five days the antibacterial efficacy of each medicament against enterococcus faecalis was checked at two different depths of 200µm and 400µm. They

concluded that 2% chlorhexidine gel is the most effective intra canal medicament against Enterococcus faecalis when compared to the other medicaments used in this study.[44]

NAZANIN ZARGAR ET AL 2018 determined the antibacterial effect of of 2% clindamycin and 100% concentration of triple antibiotic paste against enterococcus faecalis biofilm. Total of 100 root specimens were infected and divided into four groups. They were grouped as Group 1- 1000mg mL^{-1} of triple antibiotic pate, Group 2- 20 mg mL^{-1} of triple antibiotic paste, Group 3- 20 mg mL^{-1} of clindamycin, Group 4- calcium hydroxide and Group5- Methylcellulose. All medicaments were placed in the root canal for 1 week. After treatment dentin shavings were collected from 200µm and 400µm dentin depth. The number of colony forming units per mg were determined. They concluded that in order to eliminate Enterococcus faecalis biofilm 20mg/ mL^{-1}.of clindamycin or triple antibiotic paste can be used instead of full concentration. Calcium hydroxide was not as effective as its efficacy was limited to a depth of 200µm inta canal depth.[45]

VERMA R ET AL 2015 determined the antimicrobial effect of two antibacterial and two obturating pastes in dentinal tubules of primary teeth infected with enterococcus faecalis using viability stain and confocal laser scanning microscope. 28 samples were prepared. They were grouped as Group1- 1% chlorhexidine + calcium hydroxide , Group2- 2% chlorhexidine + calcium hydroxide, Group3- chlorhexidine + iodoform, and Group4- zinc oxide eugenol . All the dentinal tubules were infected with enterococcus faecalis . Two specimens from each group were taken. The specimens were subjected to antibacterial paste exposure for 1,7 and 15 days. Viability staining followed by confocal scanning microscope were used to analyze the death cell count directly inside the dentin. They concluded that 1%

chlorhexidine + calcium hydroxide (with longer contact) showed the best antimicrobial effect against enterococcus faecalis when compared to all the other groups used in this study.[46]

ARMAMENTARIUM USED

- Ultrasonic bath.
- Hot air oven
- Electrospinning
- Field emission Scanning electron microscope
- Confocal laser scanning microscope

MATERIALS USED

- Mandibular premolars
- Diamond disc
- Saline
- 5.25% Sodium Hypochlorite
- 17% EDTA
- Triple Antibiotic Paste
- Calcium hydroxide paste
- Poly vinyl pyrrolidone
- Polymer
- Fluorescent dye

INCLUSION CRITERIA

- Unilateral teeth
- Teeth with single root canal
- Teeth with circular shape canal

EXCLUSION CRITERIA

- Multiradicular teeth
- Teeth with multiple canals
- Teeth with oval or ribbon shaped canals
- Teeth with any anomalies
- Teeth with root resorption

TRIPLE ANTIBIOTIC NANOFIBER FABRICATION:

A 10 wt% Poly vinyl pyrrolidone polymer solution was prepared in hexafluoro- 2-propanol. The 3 antibiotics (metronidazole, ciprofloxacin and minocycline) were added to the Poly vinyl pyrrolidone solution at 30 wt% concentration (relative to the total PVP [600 mg] weight; ie, 180 mg of each antibiotic) and mixed together via stirring. Antibiotic-free Poly vinyl pyrrolidone (control) and the triple antibiotic–containing polymer solutions were spun into fibers at 2 mL/h, 18-cm distance, and 15–19 kV. After processing, the fibers were dried for 2 days under a vacuum to eliminate any residual solvent and stored at room temperature until it was used.

CALCIUM HYDROXIDE NANOFIBER FABRICATION:

A 10 wt% Poly vinyl pyrrolidone polymer solution was prepared in hexafluoro- 2-propanol. Calcium hydroxide powder was added to the Poly vinyl pyrrolidone solution at 10 wt% concentration (relative to the total PVP [600 mg] weight; i.e, 180 mg of calcium hydroxide) and mixed together via stirring. Medicament free Poly vinyl pyrrolidone (control) and the Calcium hydroxide containing polymer solutions were spun into fibers at 2 mL/h, 18-cm distance, and 15–19 kV. After processing, the fibers were dried for 2 days under a vacuum to eliminate any residual solvent and stored at room temperature until it was used.

SAMPLE PREPARATION:

70 mandibular lower premolar teeth stored in saline solution were used in this study. The coronal portions were cut with a 0.3mm diamond disc and the root canal length was standardized at 4× 4×1 mm radicular dentin specimens.

A biofilm was established on dentin. Gram positive, facultative anaerobic bacteria, Enterococcus faecalis, were selected, because Enterococcus faecalis is a cocci bacterium responsible for secondary infections in necrotic teeth after treatment. 70 human,caries-free, nonrestored lower premolar were used to obtain 4× 4×1 mm radicular dentin specimens. To remove the smear layer, all the specimens were placed in an ultrasonic bath containing 2.5% sodium hypochlorite followed by 17% EDTA solutions for 3 minutes each. All specimens were rinsed in saline solution for 10 minutes and autoclaved at 121°C. Next, the specimens were randomly placed into the wells of a 24-well plate containing 800 mL sterile brain-heart infusion broth and bacterial suspensions Enterococcus faecalis were inoculated into each well and allowed to grow for 7 days at 37°C in an incubator for biofilm development. The broth was changed every other day. Scanning electron microscopy (SEM) was performed to qualitatively evaluate the biofilm formation at 5^{th} day to confirm the biofilm formation. After 1 week, all specimens were rinsed for 1 minute with phosphate buffer saline solution to remove loosely bound bacterial cells. They were inoculated in all the specimens.

They were randomly divided into 5 groups based on the intracanal medicaments used

GROUP 1(N=10)-control group

GROUP 2(N=15)-Calcium hydroxide paste

GROUP 3(N=15)-Calcium hydroxide incorporated Nanofiber

GROUP 4(N=15)-Triple antibiotic paste

GROUP 5(N=15)-Triple antibiotic incorporated nanofiber

After 15 days, they were stained with the fluorescent dye to check Bacterial Viability (Live/dead bacteria). A total of 70 specimens were scanned and viewed using confocal laser scanning microscopy.

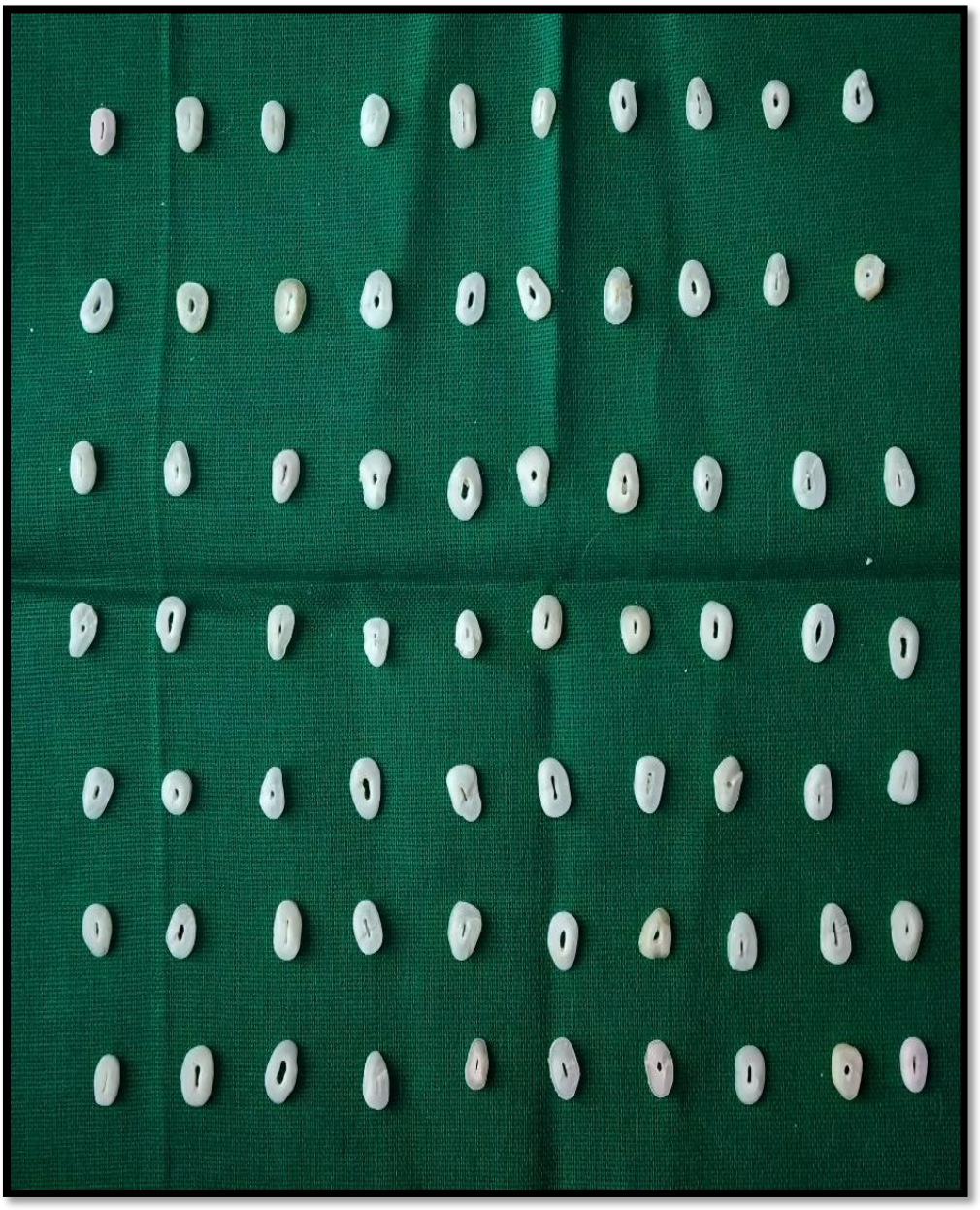


Figure 1: 70 samples of coronal third of radicular Dentin.

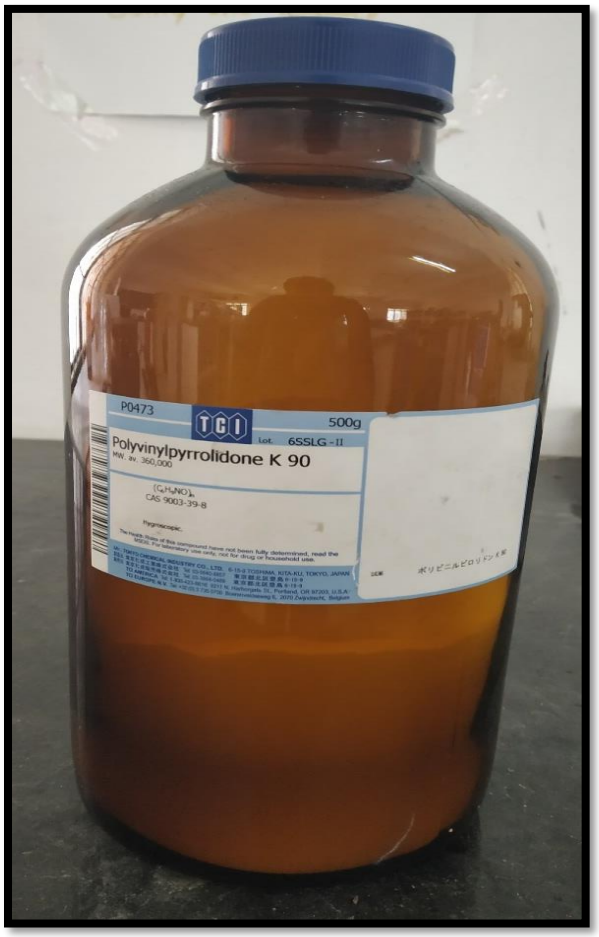


Figure 2: Polyvinylpyrrolidone

Figure 3: After dispensing of polymer and monomer

Figure 4: Magnetic stirring for triple antibiotic nanofiber for 24 hours

Figure 5: After completion of magnetic stirring for triple antibiotic nanofiber for 24 hours

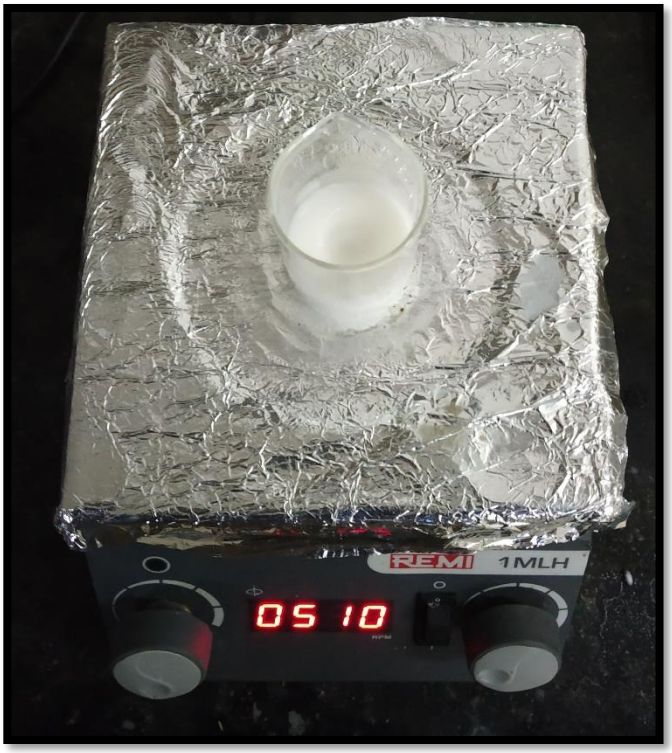


Figure 6: Magnetic stirring for calcium hydroxide nanofiber

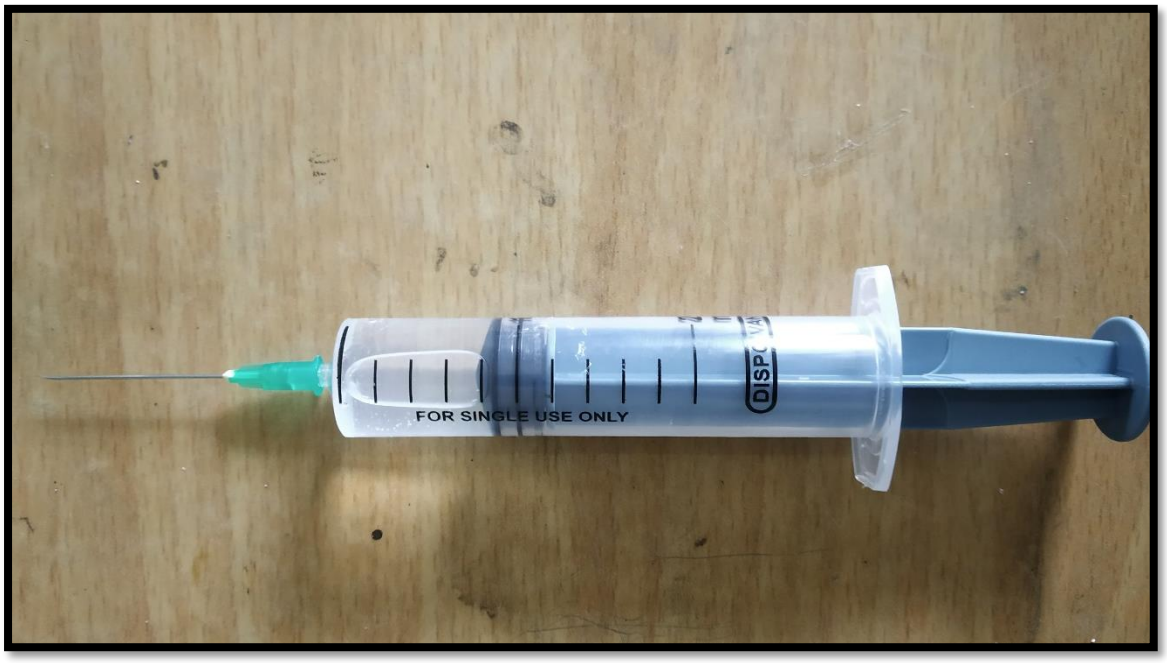


Figure 7: After 24 hours, stirred solution loaded into the syringe

Figure 8. Nano Fiber Electrospinning Unit.

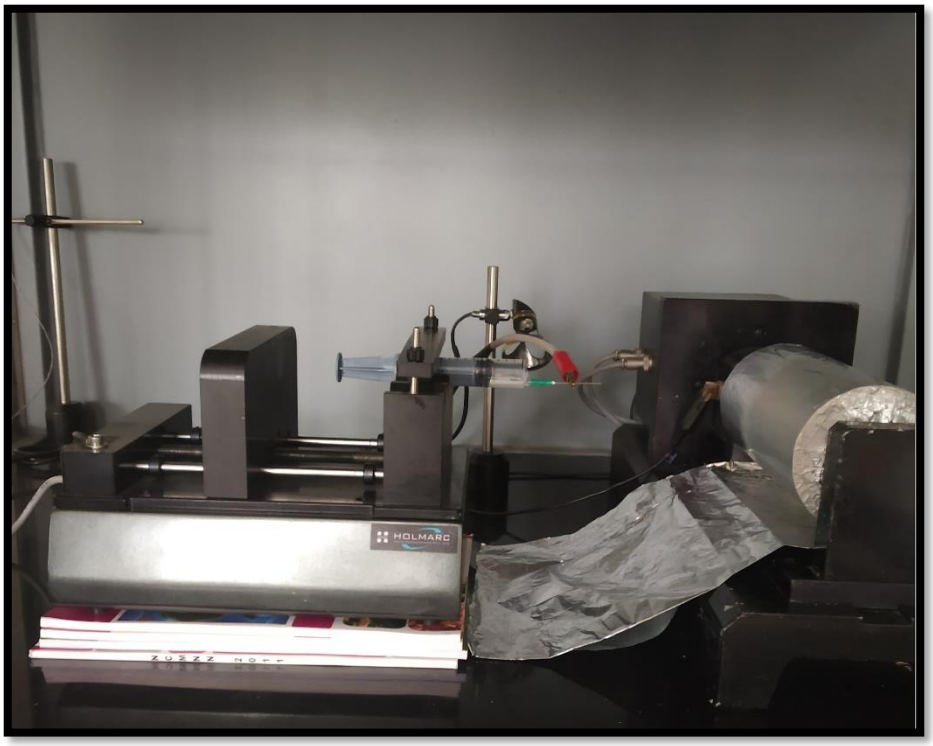


Figure 9: Placed in the electrospinning

Figure 10: After electrospinning, nanofibers are obtained

Figure 11: After removal of nanofibers.

Figure 12. Field Emission Scanning Electron Microscope.

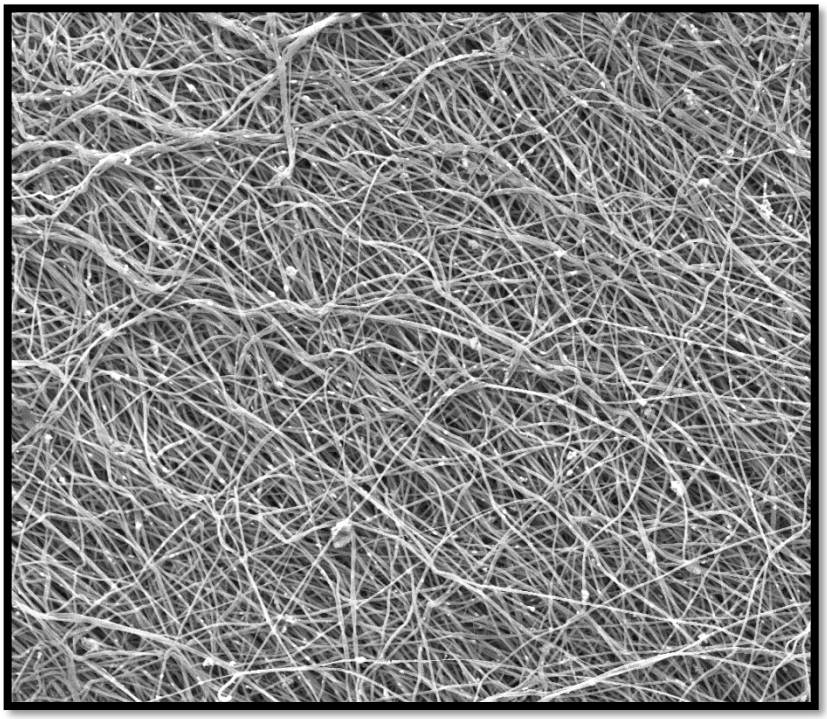


Figure 13: Nanofiber confirmed in SEM at lower magnification

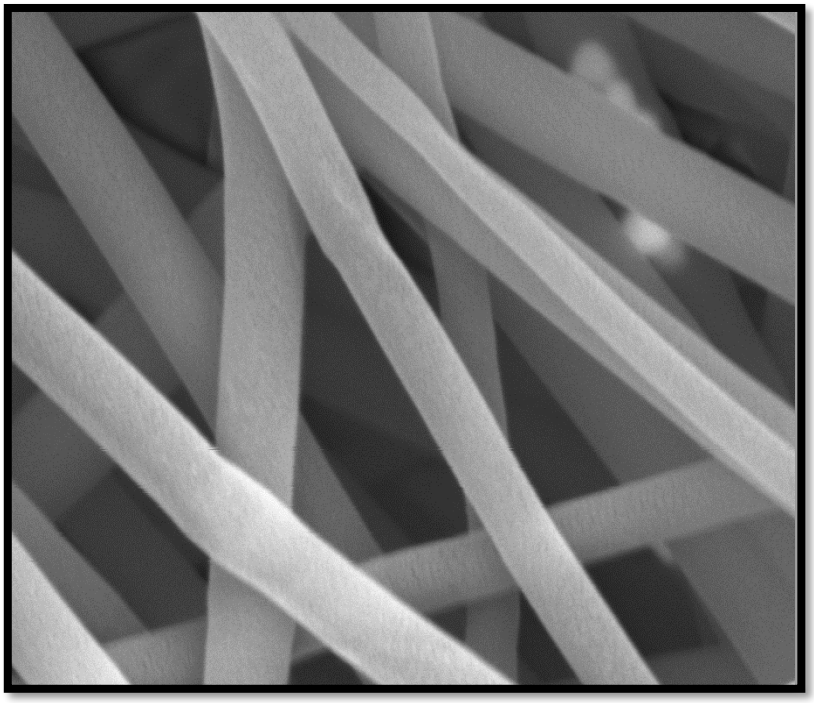


Figure 14: Nanofiber confirmed in SEM at higher magnification

Figure 15: Laminar flow chamber

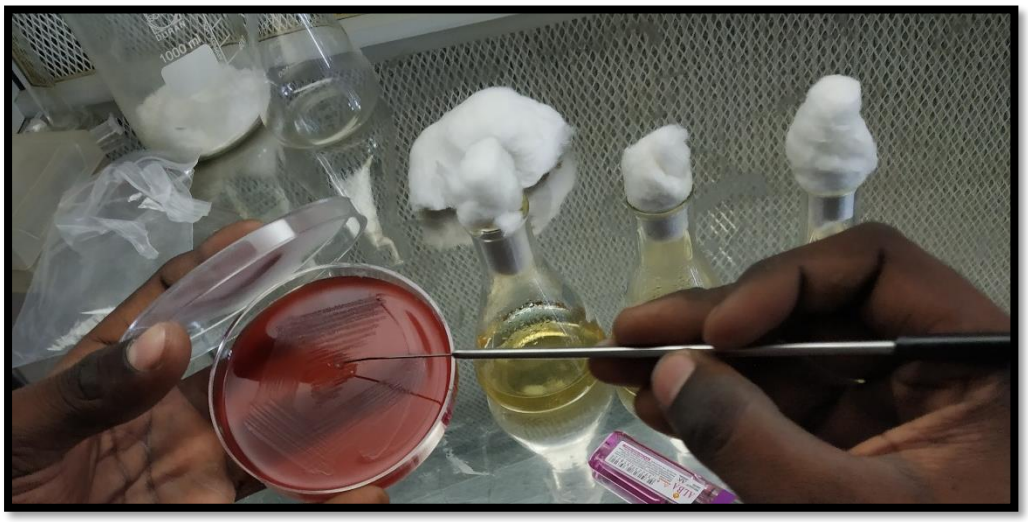


Figure 16: Enterococcus faecalis innoculation

Figure 17: placed in the incubator shaker for 2 days

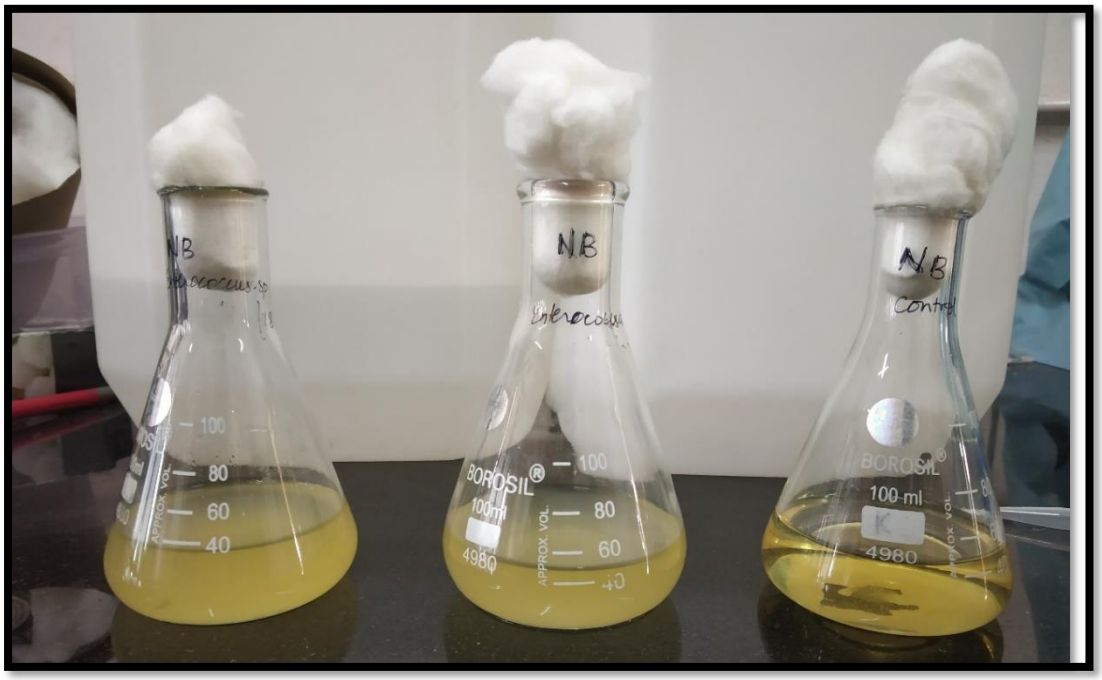


Figure 18: After 2 days, enterococcus faecalis culture

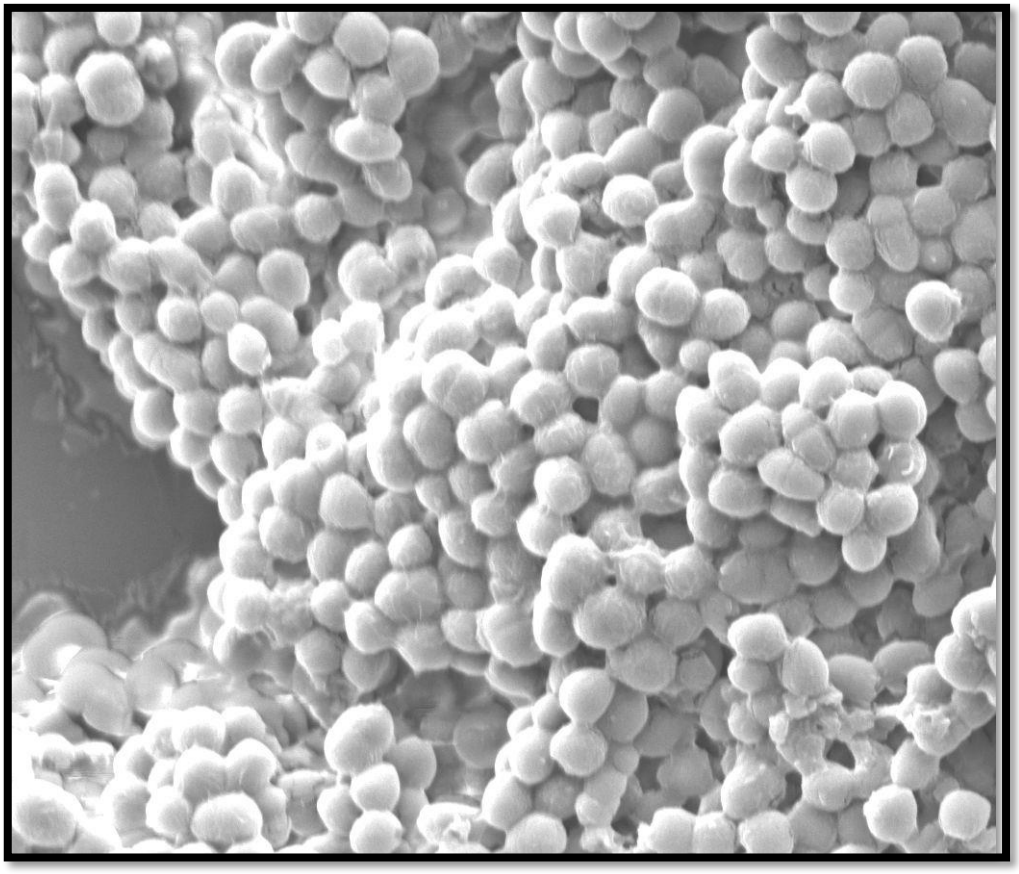


Figure 19: After 5 days, conformation of enterococcus faecalis growth in SEM

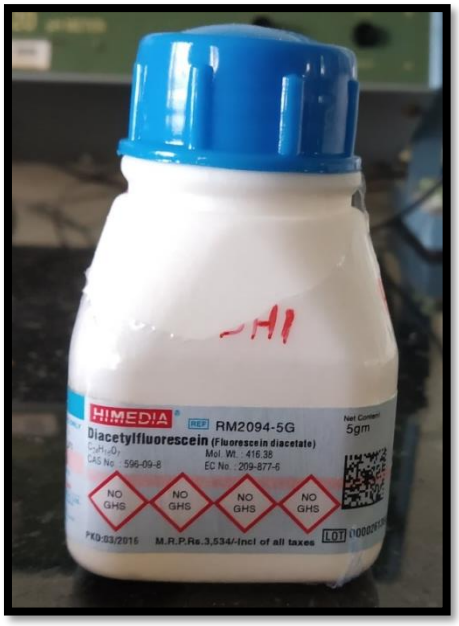


Figure 20: Fluoroscent dye.

Figure 21. Confocal Laser Scanning Microscope.

Group I- Control group (Nanofiber without medicament) Enterococcus faecalis seen in 20x magnification

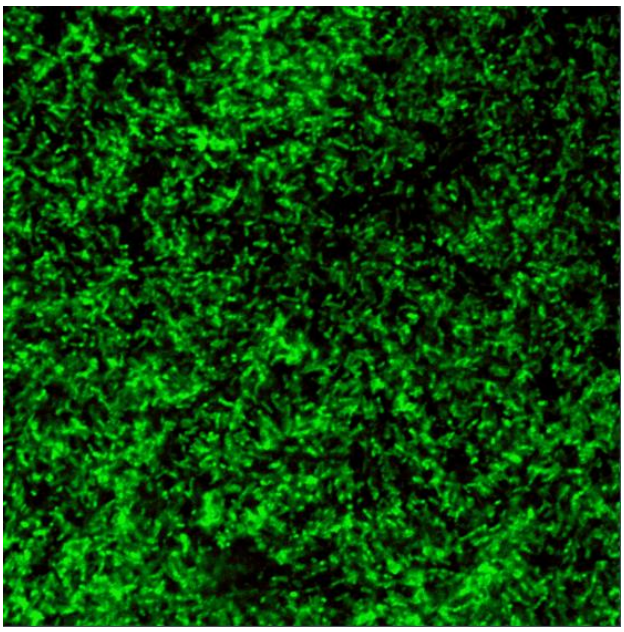


Figure 22: Live Enterococcus faecalis in control group (Group I)

Group II- Calcium hydroxide paste group, Enterococcus faecalis seen in 20x magnification

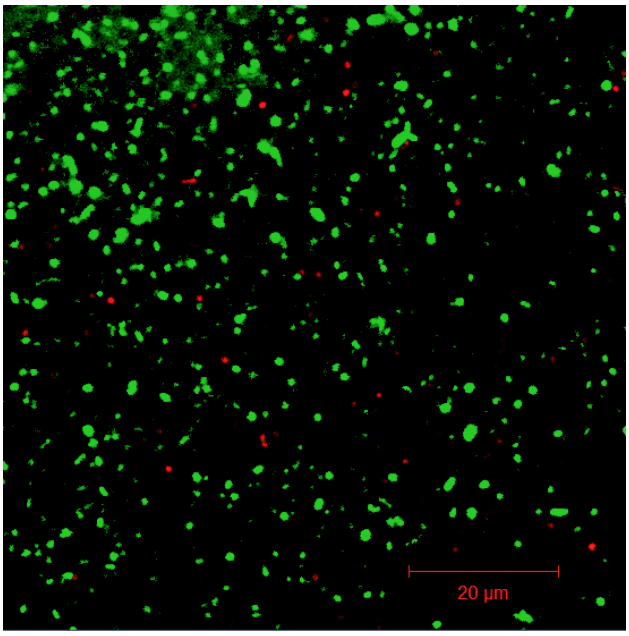


Figure 23 : Live Enterococcus faecalis in calcium hydroxide paste group (Group II)

Group III Calcium hydroxide incorporated nanofiber, Enterococcus faecalis seen in 20x magnification

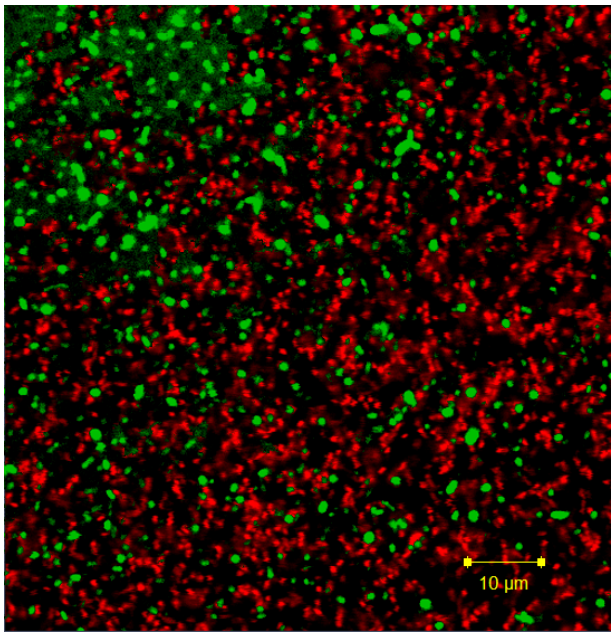


Figure 24: Live Enterococcus faecalis in calcium hydroxide nanofiber group (Group III)

Group IV- Triple antibiotic paste, Enterococcus faecalis seen in 20x magnification

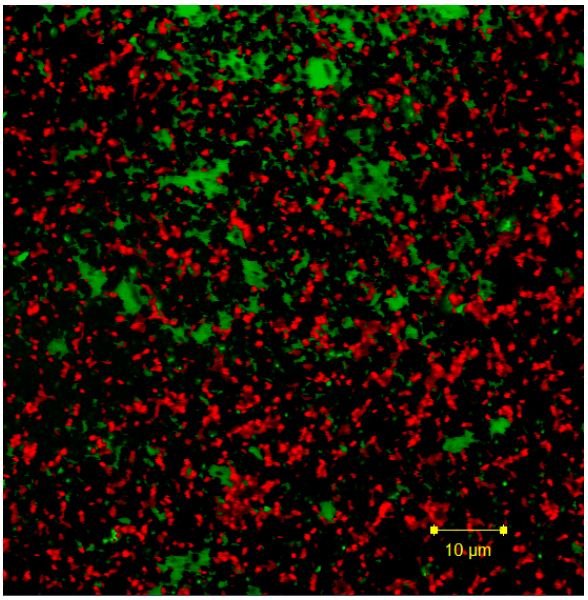


Figure 25 : Live Enterococcus Faecalis in Triple antibiotic paste group (Group IV)

Group V- Triple antibiotic incorporated nanofiber, Enterococcus faecalis seen in 20x magnification

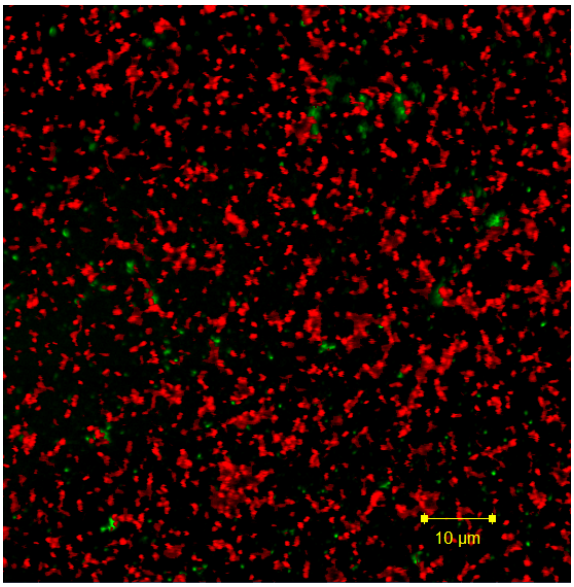


Figure 26: Live Enterococcus faecalis in Triple antibiotic nanofiber group (Group V)

STATISTICAL ANALYSIS

POST HOC TEST

Group	Total Number	Mean Value	Standard Deviation	P Value
Control (Group I)	10	94.8000	3.55278	.000
Calcium hydroxide paste (Group II)	15	85.7333	7.04543	.000
Calcium hydroxide Nanofiber (Group III)	15	72.0000	10.29563	.000
Triple antibiotic paste (Group IV)	15	25.9333	10.13809	.000
Triple antibiotic nanofiber (Group V)	15	11.2000	4.75395	.000

Table 1:Mean standard deviation for live bacteria

Post hoc test was done to evaluate the performance calcium hydroxide and triple antibiotic paste with and without nanofiber. Significant difference is seen between all the groups. Mean value is greater in Group-I followed by Group-II, Group-III, Group-IV, Group-V. (P value <0. 05, its statistically significant)

MULTIPLE COMPARISONS

TUKEY HSD

Dependent Group	Compared Group	Mean difference	Standard error	Significant difference	95% Confidence Interval	
					Lower bound	Upper Bound
Control Group (Group I)	Calcium hydroxide paste (Group II)	9.06	3.22	.049	.0272	18.1061
	Calcium hydroxide Nanofiber group (Group III)	22.8	3.22	.000	13.7606	31.8394
	Triple antibiotic paste (Group IV)	68.8	3.22	.000	68.8667	3.22167
	Triple antibiotic Nanofiber (Group V)	83.6	3.22	.000	83.60000	3.22167

Table 2: Tukey HSD for multiple comparisons of Group-I versus Group-II, Group-III, Group-IV, Group-V.

Group-I shows more live bacteria when compared to Group-II, Group-III, Group-IV and lesser live bacteria when compared to Group-V.

Dependent Group	Compared Group	Mean difference	Standard error	Significant difference	95% Confidence Interval	
					Lower bound	Upper Bound
Calcium hydroxide paste (Group II)	Control (Group I)	9.06667	3.22167	.049	18.1061	.0272
	Calcium hydroxide Nanofiber (Group III)	13.73333	2.88155	.000	5.6482	21.8184
	Triple antibiotic paste (Group IV)	59.80000	2.88155	.000	51.7149	67.8851
	Triple antibiotic Nanofiber (Group V)	74.53333	2.88155	.000	66.4482	82.6184

Table 3: Tukey HSD for multiple comparisons of Group-II versus Group-I, Group-III, Group-IV, Group-V

Group-II shows lesser live bacteria when compared to Group-I and greater live bacteria when compared to Group-III, Group-IV, Group-V.

Dependent Group	Compared Group	Mean difference	Standard error	Significant difference	95% Confidence Interval	
					Lower bound	Upper Bound
Calcium hydroxide Nano fiber (Group III)	Control (Group I)	-22.8000	3.22167	.000	-31.8394	-13.7606
	Calcium hydroxide paste (Group II)	-13.73333	2.88155	.000	-21.8184	-5.6482
	Triple antibiotic paste (Group IV)	46.06667	2.88155	.000	37.9816	54.1518
	Triple antibiotic Nanofiber (Group V)	60.80000	2.88155	.000	52.7149	68.8851

Table 4: Tukey HSD for multiple comparisons of Group-III versus Group-I, Group-II, Group-IV, Group-V

Group-III shows lesser live bacteria when compared to Group-I and Group-II and greater live bacteria when compared to Group-IV and Group-V.

Dependent Group	Compared Group	Mean difference	Standard error	Significant difference	95% Confidence Interval	
					Lower bound	Upper Bound
Triple antibiotic paste (Group IV)	Control (Group I)	-68.86667	3.22167	.000	-77.9061	-59.8272
	Calcium hydroxide paste (Group II)	-59.80000	2.88155	.000	-67.8851	-51.7149
	Calcium hydroxide Nanofiber (Group III)	-46.06667	2.88155	.000	-54.1518	-37.9816
	Triple antibiotic Nanofiber (Group V)	14.73333	2.88155	.000	6.6482	22.8184

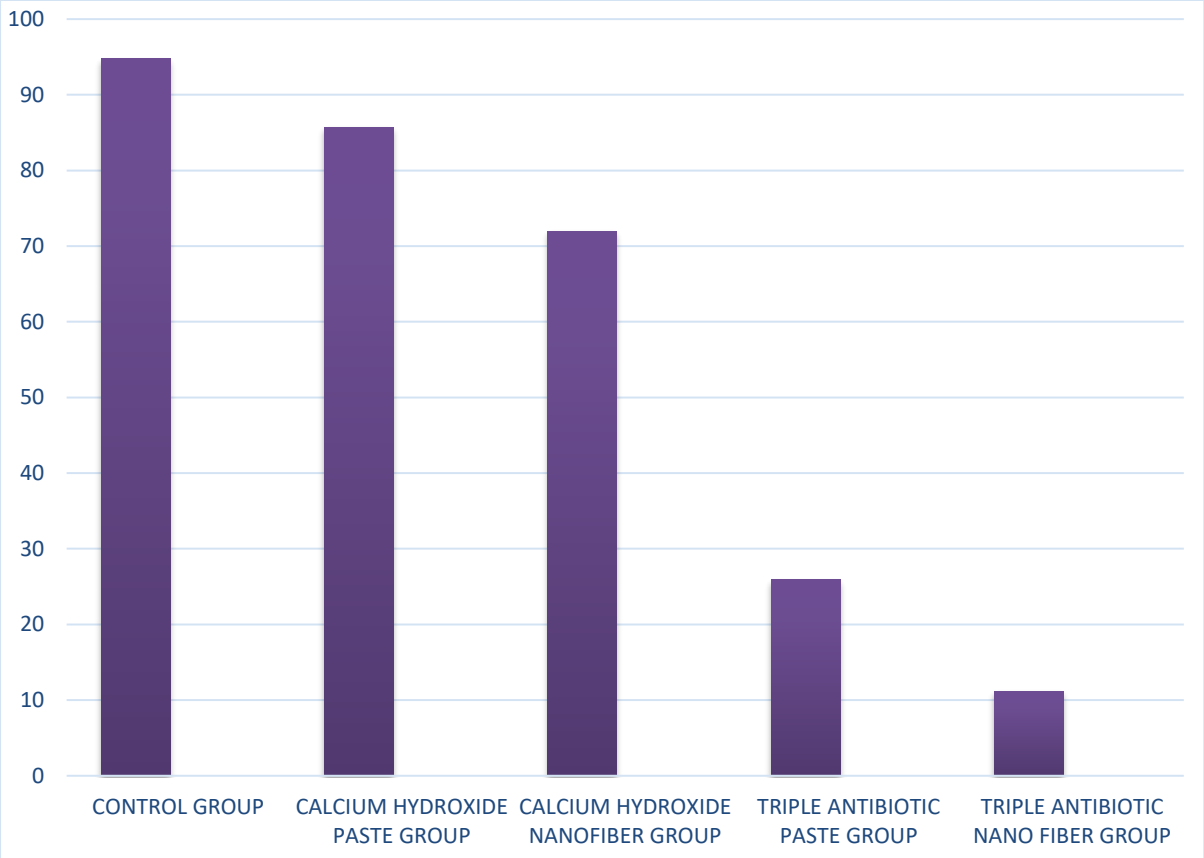
Table 5: Tukey HSD for multiple comparisons of Group-IV versus Group-I, Group-II, Group-III, Group-V

Group IV shows lesser live bacteria when compared to Group-I, Group-II, Group-III and greater live bacteria when compared to Group-V.

Dependent Group	Compared Group	Mean difference	Standard error	Significant difference	95% Confidence Interval	
					Lower bound	Upper Bound
Triple antibiotic Nano fiber (Group V)	Control (Group I)	-83.60000	3.22167	.000	-92.6394	-74.5606
	Calcium hydroxide paste (Group II)	-74.53333	2.88155	.000	-82.6184	-66.4482
	Calcium hydroxide Nanofiber (Group III)	-60.80000	2.88155	.000	-68.8851	-52.7149
	Triple antibiotic paste (Group IV)	-14.73333	2.88155	.000	-22.8184	-6.6482

Table 6: Tukey HSD for multiple comparisons of Group-V versus Group--I, Group-II, Group-III, Group-IV

Group V shows lesser live bacteria when compared to Group I, Group II, Group III, Group IV,



BAR DIAGRAM 1. LIVE BACTERIAL COUNT

Eradication of bacteria is essential for successful outcome of endodontic treatment. Thorough sterilization of the root canal and periradicular region is essential as it promotes good healing of periapical diseases in adults. The application of antibacterial medicaments to endodontic lesions is a part of the clinical procedures that may be used to sterilize lesions[47.] There is a wide range of microbes through out the root canal system that might enhance or disturb biofilm development. Commonly found bacteria in root canals are either facultative or strict anaerobes.[3] Enterococcus faecalis is one of the primary organisms that is associated with post endodontic infection. Enterococcus faecalis is a gram positive cocci that occurs singly in pairs or in short chains. It has been observed that Enterococcus faecalis has antimicrobial resistance and the ability to acclimatize to the changing environment. This enables it to survive in the root canal and cause re-infection. *Enterococcus faecalis* binds to root canal walls, accumulate, and forms communities that enables them to organize into a biofilm. It binds to dentin and proficiently invades dentinal tubules. This enables it to with stand destruction by enabling the bacteria to become 1000 times more resistant to phagocytosis, antibodies, and antimicrobials than non-bio film-producing organisms. Enterococcus faecalis withstands prolonged periods of nutritional deprivation. The antimicrobial resistance of this organism has been attributed to the formation of a protective barrier provided by the extracellular polymeric matrix. Surface adherence by bacteria to form biofilms helps in bacterial adaptation and one that is relevant to endodontic infections.[48]. It is a facultative anaerobe seen in untreated canals as a part of polymicrobial flora. It binds to dentin and proficiently invades dentinal tubules.[49]

Enterococcus faecalis present in dentinal tubules is resistant to intracanal dressings of calcium hydroxide for over 10 days. Enterococcus faecalis and candida which are commonly found in diabetic patients, are found to be greatly resistant to calcium hydroxide[8.] This organism is able to survive when calcium hydroxide is used alone as an intracanal medicaments only when the pH is lesser than 11.5. It is able to survive by maintaining pH haemostasis. It is very unlikely that a pH of 11.5 is maintained as a result of ions penetrating the cell membrane as well as the cytoplasm's buffering capacity. Therefore Calcium hydroxide is ineffective at killing enterococcus faecalis on its own.

At a pH of 11.5 or greater Enterococcus faecalis is unable to survive. However, as a result of the buffering capacity of dentin, it is very unlikely that a pH of 11.5 can be maintained in the dentinal tubules with current calcium hydroxide utilization techniques. Enterococcus faecalis has a proton pump that provides an additional means of maintaining pH homeostasis. This is achieved by "pumping" protons into the cell to lower the internal pH.[50]

Root canal infections are poly microbial in nature . As these infections are complex in nature, a combination of antibiotics is required to address the diverse microbial flora. Triple antibiotic paste is a combination of three antibiotics namely minocycline, ciprofloxacin and Metronidazole. Metronidazole is a nitro imidazole compound; selectively toxic and effective against anaerobic organisms. The Presence of redox protein reduces the nitro groups of this compound and generates free radicals that results in DNA damage and lysis of cell.

Minocycline which is primarily bacteriostatic, hinders protein synthesis by binding to a 30S ribosome in susceptible organisms. Ciprofloxain is a synthetic Fluoroquinolone with quick bactericidal action. It inhibits the enzyme bacterial DNA gyrase.

Hoshino et al recommended the use of metronidazole (500 mg) minocycline (100mg) and ciprofloxacin (200mg) at 1:1:1 ratio for 3mix formulation. Even though Triple antibiotic paste is an effective medicament, it has its own disadavantage. When used as an intra canal triple antibiotic paste medicament is shown to be most cytotoxic to human periodontal ligament fibroblasts. It promotes exacerbated inflammatory reaction in subcutaneous connective tissue, and the minocycline component causes discoloration.[51] Pereira et al conducted a study on cellular and molecular tissue response to triple antibiotic intracanal dressing and he concluded that compared to calcium hydroxide, triple antibiotic paste induced a profuse angiogenic and inflammatory response, higher vascular area, and more inflammatory cells.[52] As an intra canal medicament calcium hydroxide is not as effective as triple antibiotic paste.[53]

Calcium hydroxide is also an effective anti-endotoxin agent. However, its effect on microbial biofilms is controversial.[54] It dissociates into calcium and hydroxyl ions when it comes in contact with an aqueous solution, and the main actions of Calcium hydroxide are attributed to the effect of these ions on vital tissues, such as inducing hard tissue deposition and being antibacterial. Hydroxyl ions are responsible for the highly alkaline nature of Calcium hydroxide[55.]

Electrospinning or electrostatic spinning, a textile technology, has been utilized to fabricate antibiotic containing polymer-based nanofibers for drug delivery applications in dentistry. This mode of drug delivery is used to ablate periodontal and endodontic infections. The idea behind the use of antibiotic-containing nanofibers as a three-dimensional (3D) tubular drug delivery construct that can be placed inside the root canal system of necrotic teeth rests on the fact that the addition of low antibiotic concentrations and the slow drug release rendered by these nanofibrous constructs will be able to eradicate infection and thus generates a bacteria-free environment favorable to tissue regeneration. However in this strategy, the

antimicrobial agents could be used at a much lower concentration as they are delivered in a predictable fashion onto the dentinal walls where microbial biofilms have been detected. In electrospinning, a polymer solution/melt containing the preferred concentration of antibiotics is prepared to produce nanofibers. A high-voltage source is used to produce an electrical potential difference between the metallic needle tip and the grounded collector fixed at a set distance, which overcomes the surface tension of the fluid droplet, creating a jet. The fluid jet experiences whipping instabilities and tends to dry and produce nano to micron sized polymeric fibers. The chosen polymer solution can be incorporated with one or a combination of antibiotics, making it possible to fabricate fibers with a narrow or wide spectrum of action (e.g., ciprofloxacin, metronidazole, and minocycline, among others) that have been shown to inhibit the growth of endodontic pathogens.[56]

In this study it has been observed that all the four intra canal medicaments do not consistently and completely eradicate the enterococcus faecalis biofilm in the root canal. The control Group (nanofiber) without medicament showed more live cells than the other four groups. Enterococcus faecalis showed successful biofilm formation when viewed under field emission scanning electron microscope. Confocal laser scanning electron microscope showed that a dense population of viable bacteria adhered to dentin. Triple antibiotic incorporated nanofibers (Group 5) showed nearly complete elimination of viable bacteria on the dentin surface when observed under the confocal laser scanning microscope. Triple antibiotic incorporated nanofiber group showed significantly higher eradication of Enterococcus faecalis biofilm, when compared with the triple antibiotic paste group, calcium hydroxide paste group, and calcium hydroxide incorporated nanofiber group.

On inter group comparsion control group showed lesser eradication of Enterococcus faecalis biofilm than the calcium hydroxide paste group, calcium hydroxide incorporated nanofiber group, triple antibiotic paste group, triple antibiotic incorporated nanofiber group.

When comparing the calcium hydroxide group with the other groups, calcium hydroxide showed greater erradication of Enterococcus faecalis than control group and lesser eradication of Enterococcus faecalis than calcium hydroxide incorporated nanofiber group, triple antibiotic paste group, triple antibiotic incorporated nanofiber groups.

When comparing the calcium hydroxide incorporated nanofiber group with the other groups, the calcium hydroxide incorporated nanofiber group showed greater eradication of Enterococcus faecalis than control group and calcium hydroxide group and smaller than triple antibiotic paste group, triple antibiotic incorporated nanofiber group.

When comparing the triple antibiotic paste group with other the groups, triple antibiotic paste group showed greater erradication of Enterococcus faecalis biofilm than the control group, calcium hydroxide group, calcium hydroxide incorporated nanofiber group. When compared with the triple antibiotic incorporated nanofiber group, triple antibiotic paste group showed lesser erradication of Enterococcus faecalis.

The results of the present study coincides with studies conducted by Madhubala et al and DeLucena et al in which the triple antibiotic paste group showed higher antibacterial effects than calcium hydroxide on Enterococcus faecalis.

Shojaee NS and Motamedifer et al concluded that Triple antibiotic paste has Minocycline which is the most effective component against Enterococcus faecalis. Bactericidal effect of calcium hydroxide against Enterococcus faecalis was lower when compared to the triple antibiotic paste.

Mohammed Ali and Mozayeni et al concluded that triple antibiotic paste including Metronidazole, Ciprofloxacin, Minocycline had an appropriate effect on Enterococcus faecalis than calcium hydroxide.

When comparing the triple antibiotic incorporated nanofiber group with the other groups,the triple antibiotic incorporated nanofiber group showed greater eradication of Enterococcus faecalis than control group, calcium hydroxide paste group, calcium hydroxide incorporated nanofibergroup and triple antibiotic paste group.

SUMMARY

Seventy mandibular single rooted teeth were taken and the coronal portions were cut with a 0.3mm diamond disc and standardized at 4× 4×1 mm radicular dentin specimens and the specimens were inoculated with Enterococcus faecalis and two intracanal medicaments such as calcium hydroxide and triple antibiotic paste with and without nanofiber were placed inside the specimen and kept for 14 days. The live bacteria were evaluated using confocal laser scanning electron microscope.

The findings of the present study is summarized as follows

1. There was a statistically significant difference between all the test groups.
2. Calcium hydroxide nanofiber group has statistically significant difference when compared with calcium hydroxide paste group.
3. Triple antibiotic nanofiber group has statistically significant difference when compared with triple antibiotic paste group.
4. Triple antibiotic paste group has statistically significant difference when compared with calcium hydroxide paste group.
5. Triple antibiotic paste group has statistically significant difference when compared with calcium hydroxide nanofiber group.
6. Triple antibiotic nanofiber group has statistically significant difference when compared with calcium hydroxide nanofiber group.

Within the limitations of the study, it was concluded that triple antibiotic nanofibers are more effective against enterococcus faecalis followed by triple antibiotic paste, calcium hydroxide nanofiber and calcium hydroxide. Clinically, Triple antibiotic paste is more effective than calcium hydroxide and the nanofibers increase the efficacy of the intracanal medicament used by its effective drug delivery property.

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